

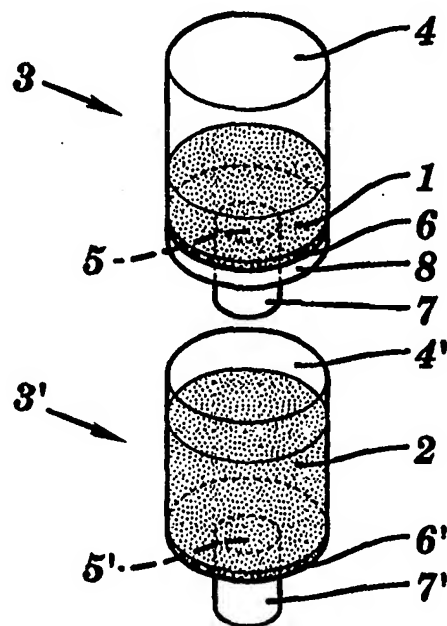
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/23997 <b>(22) International Filing Date:</b> 10 November 1998 (10.11.98) <b>(30) Priority Data:</b> 60/065,387 12 November 1997 (12.11.97) US <b>(71) Applicant:</b> BIOSEPPA INC. [US/US]; 111 Locke Drive, Marlborough, MA 01752 (US). <b>(72) Inventors:</b> COFFMAN, Jonathan, L.; 2 Spinnaker Lane, Haverhill, MA 01830 (US). WOODS, Ernest, J., III; 9 Plumbly Road, Upton, MA 01568 (US). LU, Tze, Hong; Apartment 11, 20 Williamsburg Court, Shrewsbury, MA 01545 (US). <b>(74) Agents:</b> CLEMENT, Candice, J. et al.; Heslin & Rothenberg, P.C., 5 Columbia Circle, Albany, NY 12189 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	

**(54) Title:** DEVICE AND METHODS FOR PURIFYING BIOLOGICAL MOLECULES USING LOW VOLUMES OF CHROMATOGRAPHIC AND ADSORPTIVE MEDIA

**(57) Abstract**

A device and methods for purification of biological molecules are disclosed. The device comprises a volume, 30  $\mu$ L or less, of an adsorptive media (1) disposed over a volume, 100  $\mu$ L or less, of a chromatographic media (2). Preferably, the adsorptive media is an ion exchange media and the chromatographic media is a desalting media. The media may be packed in a single housing or in separate housings (4, 4'), which due to the small volume of media, can be an individual well in a multiwell device. Methods for purification of biological molecules using the device are also disclosed. In accordance with the principles of the invention, a biological sample is introduced into the device, wherein molecules of interest are adsorbed onto the adsorptive media, then a volume of elution buffer, preferably less than or equal to one-third the volume of the second chromatographic media, is used to elute the desired molecules from the adsorptive media, wherein the resulting elutant passes into the second chromatographic media for collection.



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# DEVICE AND METHODS FOR PURIFYING BIOLOGICAL MOLECULES USING LOW VOLUMES OF CHROMATOGRAPHIC AND ADSORPTIVE MEDIA

## Field of Invention

The present invention relates to the processing of biological samples, and more particularly, to a device and methods for purifying biological/chemical molecules using small volumes of media.

## Background of Invention

There are many devices and methods used in the purification of biological or chemical molecules where an adsorptive media is packed into a well-type device and a sample comprised of the molecules of interest and other impurities is loaded into the device, wherein the molecules of interest are adsorbed onto the adsorptive media while the remaining portions of the sample pass therethrough. The adsorptive media is then washed to remove any loosely bound molecules. An appropriate elution buffer is then introduced to desorb the bound molecules of interest, wherein the molecules are then collected in the elution buffer.

Prior to the present invention, this technique involved the use of relatively large volumes, of adsorptive media. The majority of research involving biological samples, however, typically involves the use of small volume receptacles, such as syringes and multiwell culture, filter or collection plates. Such plates are well known in the art and are exemplified, for example, by those described in U.S. Patents 3,111,489; 3,540,856; 3,540,857; 3,540,858; 4,304,865; 4,948,442; and 5,047,215. A typical example is a standardized 96-well plate which provides 96 depressions or cylindrical wells of about 0.6 cm in diameter and 1.3 cm deep. Other examples include a 384-well, an 864-well and a 1536-well plate. While each individual well of a 96-well plate has a capacity of about 400  $\mu$ L, the capacity of a well of a 384-well plate is only about 100  $\mu$ L, and for an 864-well plate, only about 40  $\mu$ L.

It is desired to purify biological molecules in multiwell plates or in other miniaturized devices. This miniaturization presents constraints to the design and use of such devices which are not immediately obvious. Therefore, to miniaturize a device that will perform a primary separation on an adsorptive media, then elute with an elution buffer, and then separate the elution buffer from the biological molecules of interest and place the molecules into any other buffer of interest is not obvious.

The present invention allows the researcher to adequately and efficiently perform chromatographic separation and purification of molecules of interest in small volume receptacles, such as for example, individual wells of a multiwell plate.

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### Summary of Invention

In one embodiment, the present invention relates to a device for purification of biological molecules from a biological sample comprised of the molecules of interest and other impurities. The device is essentially a pair of housings, each having an inlet and an outlet, the outlet having a porous material, such as a porous frit or membrane, disposed there over; and each having a volume of a chromatographic media packed therein. The first housing includes a packed volume of 30  $\mu$ L or less of an adsorptive media, preferably an ion exchange media, and the second housing includes a packed volume of 100  $\mu$ L or less of a second chromatographic media, preferably a desalting media. The housings are aligned such that a material exiting the outlet of the first housing passes into the inlet of the second housing. In a preferred embodiment of the device, the first and second housings are corresponding wells of a multiwell plate, such as a 96-well, a 384-well, an 864-well, or a 1536-well plate.

Accordingly, one embodiment of the present invention is a device for purification of biological molecules, the device comprising:

a first housing having an inlet in an upper surface and an outlet in a lower surface, and wherein a porous material is disposed over the outlet;

a volume of an adsorptive media packed in the first housing, over the porous material and wherein a packed volume of the adsorptive media is 30  $\mu$ L or less;

a second housing having an inlet in an upper surface and an outlet in a lower surface, and wherein a porous material is disposed over the outlet; and

a volume of a second chromatographic media packed in the second housing, over the porous material, and wherein a packed volume of the second media is

5 100  $\mu$ L or less;

wherein the outlet of the first housing is aligned with the inlet of the second housing such that a material exiting the outlet of the first housing passes into the inlet of the second housing.

In one embodiment of the device, the packed volume of the adsorptive media is less  
10 than 15  $\mu$ L and the packed volume of the second chromatographic is less than 75  $\mu$ L, and in another embodiment, less than 10  $\mu$ L and less than 30  $\mu$ L, respectively.

The device may further include means for sealing the first housing to the second housing, to prevent contamination of material exiting the first housing and entering the second housing. Such contamination can occur as a result of impurities being drawn in  
15 from the ambient atmosphere, or as a result of cross-contamination from neighboring wells when the housings are multiwell devices.

In accordance with the principles of the present invention, the volume of adsorptive media and the volume of a second chromatographic media may be packed within the same housing. Accordingly, another embodiment of the invention is a device for purification of  
20 biological molecules, the device comprising:

a housing having an inlet in an upper surface and an outlet in a lower surface, and a porous material disposed over the outlet; and

a volume of chromatographic media packed in the housing, the chromatographic media comprising:

25 a packed volume of 30  $\mu$ L or less of an adsorptive media and  
a packed volume of 100  $\mu$ L or less of a second chromatographic media.

Preferably, the packed volume of the adsorptive media is less than 15  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 75  $\mu\text{L}$ , and more preferably, less than 10  $\mu\text{L}$  and less than 30  $\mu\text{L}$ , respectively. Preferably, the adsorptive media is an ion exchange media and the second chromatographic media is a desalting media.

5           The present invention also relates to methods involving the use of the various embodiments of the device of the present invention for the purification of biological molecules. In a preferred aspect of the present invention, the devices of the present invention are used for the purification of nucleic acids, including polymeric nucleic acids and DNA molecules.

10           Accordingly, one aspect of the present invention is a method for purification of biological molecules, the method comprising the steps of:

              preequilibrating a device constructed in accordance with the principles of the present invention with a buffer;

              loading the preequilibrated device with a biological sample comprised of biological

15           molecules of interest and other impurities, wherein said molecules of interest are adsorbed onto the adsorptive media;

              washing the loaded device with one or more wash buffers;

              eluting the molecules of interest from said adsorptive media with a volume of an elution buffer; and

20           collecting the resulting elutant, wherein said elutant comprises the molecules of interest.

In a preferred aspect of this method, the volume of elution buffer is less than or equal to one-third the volume of the second chromatographic media.

25           The step of eluting may comprise introducing the elution buffer into the loaded device and then drawing the elution buffer through the adsorptive media, thereby eluting the molecules of interest, and then drawing the resulting elutant through the second

chromatographic media. In one aspect, after the elution buffer has been introduced, the loaded device is centrifuged, to accomplish the elution of molecules and to draw the elutant through the second chromatographic media.

In another aspect, the method is further characterized in that the device includes  
5 sealing means, such as a gasket disposed on a bottom surface of the first housing or an O-ring disposed on the upper surface of the second housing, for forming a seal between the first and second housings, such that elution of the molecules of interest and drawing the resulting elutant through the second chromatographic media may be accomplished by such means as a vacuum, a pressure differential or electro-osmosis.

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#### Brief Description of the Drawings

Figure 1 is a schematic depiction of a device of the present invention wherein the adsorptive media and the second chromatographic media are packed in separate housings;  
and

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Figure 2 is a schematic depiction of the purification of a biological sample in accordance with the principles of the present invention, wherein arrow X indicates the flow of material through a two housing device of the present invention.

#### Detailed Description of the Invention

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The principles of the present invention are hereinafter described with reference to specific embodiments. It is not, however, intended that the invention be limited to these  
embodiments.

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In the processing of biological molecules, after doing a primary purification on an ion exchange, reversed-phase or other adsorptive media, it is desirable to remove the buffer that was used to desorb the biological molecules of interest. This can be accomplished by binding to a second adsorptive media, or by using a second chromatographic media, such as size exclusion desalting media.

The volume of the desalting media that can be used is constrained by the volume of many multiwell plates to be 100  $\mu\text{L}$  or less. It is not immediately obvious, but the volume of the desalting media in turn constrains the volume of the elution buffer used, since most size exclusion desalting media can only desalt a volume of less than one-third the volume of the desalting media. A 100  $\mu\text{L}$  volume of desalting media therefore can desalt a volume of only approximately 30  $\mu\text{L}$  or less. Further, it is difficult to elute with a volume of liquid that is smaller than the volume of the adsorptive bed, since the adsorptive bed should be covered effectively with the volume of the elution buffer. Thus, the volume of the first adsorptive media is constrained to be less than or equal to the volume of the elution buffer, which is constrained to be less than or equal to one third of the volume of the desalting media. Thus, the volume of the desalting media is constrained by the volume of the multiwell plate, which in turn constrains the volume of the elution buffer, which in turn constrains the volume of the first adsorptive media. This means that wherein the volume of the desalting media is about 100  $\mu\text{L}$  or less, the volume of the elution buffer is about 30  $\mu\text{L}$  or less and the volume of the first adsorptive media is about 30  $\mu\text{L}$  or less.

As shown in Figure 1, the present invention relates to a device for separation or purification of biological molecules of interest, wherein a small packed volume, 30  $\mu\text{L}$  or less, of an adsorptive media (1) is disposed over a packed volume, 100  $\mu\text{L}$  or less, of a second chromatographic media (2). The adsorptive media is preferably an ion exchange media, but may also comprise media such as, for example, affinity media, normal phase media, reversed-phase media, metal chelating media, and hydroxyapatite media. The second chromatographic media is preferably a desalting media, but may also comprise media such as size exclusion media, silica binding media, hydrophobic-interaction media, and reversed-phase media. In preferred embodiments of the device, the packed volume of the adsorptive media will be less than 15  $\mu\text{L}$  and the packed volume of the second chromatographic media will be less than 75  $\mu\text{L}$ , and more preferably, less than 10  $\mu\text{L}$  and less than 30  $\mu\text{L}$ , respectively.

The two media may be packed in a single housing or in separate housings. Due to the small packed volumes of the media, a housing may comprise an individual well of a



multiwell device, such as, for example, a 96-well, a 384-well, an 864-well, or a 1536-well multiwell plate. Referring again to Figure 1, each housing (3,3') includes an inlet (4,4') in an upper surface and an outlet (5,5') in a lower surface. Prior to packing the media into the housing a porous material (6,6') is disposed over the outlet to prevent the media from  
5 seeping out of the outlet during packing. The porous material can comprise, for example a porous frit or membrane, such as those well known in the art, including, but not limited to, polypropylene frits having pore sizes ranging from about 0.45  $\mu\text{m}$  to about 50  $\mu\text{m}$ , and preferably from about 1  $\mu\text{m}$  to about 20  $\mu\text{m}$  (such frits are commercially available from Porex Technologies, Fairburn, GA USA).

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When the media are packed into separate housings, the housings are aligned in such a manner wherein the outlet (5) of the first housing corresponds to the inlet (4') in the second housing, wherein a material exiting the first device passes directly into the second device. Various means may be included in the device for efficiently accomplishing the transfer of  
15 material between the two housings. For instance, the housing may be constructed to include a drip director (7,7') depending from its lower surface such that material exiting the first housing will be directed into the inlet (4') of the second housing when the two housings are in alignment.

In addition, the device may include means for forming a seal between the two  
20 housings when they are in proper alignment. This means could be a gasket (8), disposed on the underside of the lower surface of the first housing, wherein the gasket covers the surface surrounding the outlet. When the housings are corresponding wells in separate multiwell plates, the gasket covers the entire surface adjacent to the outlets of each well in the first plate, preventing cross-contamination between the wells as material is transferred from the  
25 first plate to the second. The sealing means may also comprise an O-ring or rim gasket (not shown), that is disposed on the upper surface of the second housing, either as an element of the housing, or as a structure separate from the housings, in which case it would be placed atop the second housing prior to alignment with the first housing. In this manner, the O-ring functions to seal the interior region between the two housings from contamination by

impurities present in the ambient atmosphere. The gasket and the O-ring or rim gasket may, for example, be fabricated of a closed-cell foam material or rubber.

In yet another embodiment, the sealing means can comprise an interference fit between the outlet or drip director of the first housing and the inlet of the second housing. In this embodiment, the outlet or drip director of the first housing would be fabricated to tightly mesh with the inlet of the second housing when the two are in alignment.

It is an aspect of the invention to utilize the different embodiments of the device in methods for purification of biological molecules of interest from any number of biological samples. Such samples may include, but are not limited to, a crude plasmid solution, such as that obtained from potassium acetate precipitation according to known methods (*see, e.g., Molecular Cloning*, Sambrook, Fritsch, Maniatis, Cold Spring Harbor, Plainview, NY (1989)), and plasmid or genomic DNA prepared using a variety of other methods well known in the art. Plasmid samples can include, for example, a pUC 19 cell lysate or a  $\beta$  galactosidase ( $\beta$  gal) cell lysate as in the examples described in detail below.

In accordance with the methods of the present invention, a device, constructed in accordance with the principles of the invention, is preequilibrated with a buffer solution, and then loaded with a biological sample or mixture comprising biological/chemical molecules of interest and other undesired impurities, wherein the molecules of interest become adsorbed onto the adsorptive media. The loaded device is then washed with one or more wash buffers to remove any loosely bound molecules. A predetermined volume of an elution buffer is then passed through the device, eluting the molecules of interest from the adsorptive media. The elutant then passes through the second chromatographic media, preferably a desalting media, wherein the biological molecules of interest are collected.

Figure 2 depicts the alignment of a first housing (3), containing adsorptive media (1) and a second housing (3'), containing a second chromatographic media (2), and arrow X illustrates the flow of material through the device, into a collection well (9).

In accordance with the principles of the invention, the volume of the elution buffer will be less than or equal to one-third the packed volume of the second chromatographic media. Thus, for example, the adsorptive media could be an ion exchange media in a packed volume of 30  $\mu$ L, the second chromatographic media could be a desalting media in a packed volume of 100  $\mu$ L, wherein the volume of elution buffer would be about 30  $\mu$ L.

The step of eluting the molecules of interest from the adsorptive media may comprise centrifuging the device, wherein the molecules of interest are desorbed from the adsorptive media and the resulting elutant is drawn through the second chromatographic media. Other methods known in the art may also be utilized. For example, a vacuum may be introduced at the lower surface of the second chromatographic media, a pressure differential may be applied between the upper surface of the adsorptive media and the lower surface of the second chromatographic media, and different voltages may be applied to the upper surface of the adsorptive media and the lower surface of the second chromatographic media, such that electro-osmosis occurs. In the latter methods, when the device comprises two separate media-packed housings, it is necessary that the device further include a sealing means to form a hermetic seal between the housings. Such sealing means can include, for example, a gasket disposed on the bottom surface of the first housing; an O-ring or rim gasket disposed on the upper surface of the second housing, either as an element of the second housing or as a structure separate from the two housings; and an interference fit between the outlet of the first housing and the inlet of the second housing.

Media suitable for use as the adsorptive media in the device and methods of the present invention include, but are not limited to, media such as reversed-phase media, ion exchange media, normal phase media, hydroxyapatite media, metal chelating media, affinity media, and any other media which binds molecules in one buffer, and releases molecules in a different buffer. For example, adsorptive media may be chosen from PLASMIDEX™ 20 $\mu$  (commercially available from BioSeptra, Inc., Marlborough, MA USA), Q FAST FLOW (commercially available from Pharmacia Biotech, Uppsala, Sweden), QIAGEN PLASMID MEDIA (commercially available from Qiagen Inc., Chatsworth, CA USA).

Media suitable for use as the second chromatographic media in the device and methods of the present invention include, but are not limited to, media such as desalting media, reversed-phase media, silica binding media, hydrophobic-interaction media, and size exclusion media. Size exclusion chromatography media include, but are not limited to, ACA media, such as ACA 34, ACA 44, ACA 54, and ACA 200 (commercially available from BioSeptra, Inc., Marlborough, MA USA), GF05 and GF2000 (commercially available from BioSeptra, Inc., Marlborough, MA USA), Sephacryl media, such as S400, S500, and S-1000 (commercially available from Pharmacia Biotech, Uppsala, Sweden), Superdex media, such as Superdex 75 and Superdex 200 (commercially available from Pharmacia Biotech, Uppsala, Sweden), and the like.

Preequilibration buffers suitable for use in the methods of the present invention include, but are not limited to, 50 mM Tris pH 8, 0 to 2M NaCl or 50 mM MOPS 0-2 M NaCl, or other buffer/salt combinations. In general, the pH of the preequilibration buffer will be in the range of about pH 1 to about pH 12. A preferred preequilibration buffer is 500 mM NaCl, 50 mM Tris pH 8.

Buffers suitable for use as a wash buffer in the methods of the present invention may be selected from salt buffers, wherein the choice of salt is dictated by what salt could elute the impurities but not the desired product, e.g., plasmids, in a particular sample. Examples include, but are not limited to, 500 mM to 2 M NaCl in a buffer such as Tris pH 8, Tris pH 7, MOPS, or other suitable buffers.

Buffers suitable for use as an elution buffer in the methods of the present invention may include, for example, high salt buffers, such as NaCl, KCl,  $MgCl_2$ , or  $CaCl_2$ , buffered with buffers known to those skilled in the art, such as Tris buffer, MOPS buffer, acetate buffer, phosphate buffer, and the like.

Housings for use in the device and methods of the present invention can be readily determined by the skilled artisan. Such housings may include, but are not limited to, syringe-like devices, tubes, and wells of a multiwell plate, including, but not limited to

4-well, 16-well, 96-well, 364-well, 864-well and 1536-well multiwell plates.

The invention is further defined by reference to the following examples that describe in detail particular aspects of the present invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without  
5 departing from the purpose and scope of this invention. The following examples are illustrative only and should in no way limit the scope of the present invention.

### EXAMPLE 1

#### Controls

Promega spin column of each plasmid; IPA precipitation of each plasmid

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#### Plasmid Samples

pUC 19 - 2.2 g of cell paste;  $\beta$  galactosidase ( $\beta$  gal) - 1.6 g of cell paste

#### Resuspension

The pUC 19 was resuspended in 29.2 mL resuspension buffer (200  $\mu$ g/mL RNase A, 200 units per prep RNase ); yielding 10.0 mg cell paste/well.

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The  $\beta$  gal was resuspended in 28 mL resuspension buffer (200  $\mu$ g/mL RNase A, 200 units per prep RNase ), yielding 7.6 mg cell paste /well.

133  $\mu$ L of each plasmid preparation was decanted into four 96-well plates, with only one plasmid per 96-well plate.

#### Lysis

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133  $\mu$ L of 0.2 M NaOH, 1% SDS was added to each well in pUC 19 plate 1 with a multiwell pipettor. The plate was shook gently after the addition of lysis solution to each row. This was incubated for 5 minutes.

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133  $\mu$ L of 0.2 M NaOH, 1% SDS was added to each well in pUC 19 plate 2 with a multiwell pipettor. The plate was shook gently after the addition of lysis solution to each row. This was incubated for 5 minutes.

133  $\mu$ L of 0.2 M NaOH, 1% SDS was added to each well in  $\beta$  gal plate 3 with a multiwell pipettor. The plate was shook gently after each addition of lysis solution to each row. This was incubated for 5 minutes.

5 133  $\mu$ L of 0.2 M NaOH, 1% SDS was added to each well in  $\beta$  gal plate 4 with a multiwell pipettor. The plate was shook gently after each addition of lysis solution to each row. This was incubated for 5 minutes.

#### Neutralization

133  $\mu$ L of 1.32 M KAc (Promega) was added to each well of pUC 19 plate 1 with a multiwell pipettor. The plate was shook gently after each addition of neutralization solution to each row. This was incubated for 5 minutes.

133  $\mu$ L of 1.32 M KAc (Promega) was added to each well of pUC 19 plate 2 with a multiwell pipettor. The plate was shook gently after each addition of neutralization solution to each row. This was incubated for 5 minutes.

15 133  $\mu$ L of 1.32 M KAc (Promega) was added to each well of  $\beta$  gal plate 3 with a multiwell pipettor. The plate was shook gently after each addition of neutralization solution to each row. This was incubated for 5 minutes or so.

133  $\mu$ L of 1.32 M KAc (Promega) was added to each well of  $\beta$  gal plate 4 with a multiwell pipettor. The plate was shook gently after each addition of neutralization solution to each row. This was incubated for 5 minutes or so.

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#### Filtration

All of each well of plate 1 was added to a 96-well filter plate, equipped with a soft (3 durometer) gasket, 10  $\mu$ m polypropylene membrane, 800  $\mu$ L capacity. Well A1 of the 96-well filter plate was applied to well A1 of a 384-well plate, which was filled with 15  $\mu$ L of Q Hyper D 20  $\mu$ m, lot 7195, ion exchange media and 50 mM Tris pH 7m 500 mM NaCl.

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All of each well of plate 2 was added to a 96-well filter plate. Well A1 of the 96-well plate was applied to A2 of 384-well plate, which was filled with 15  $\mu$ L of Q Hyper D 20  $\mu$ m, lot 7195 and 50 mM Tris pH 7m 500 mM NaCl.

All of each well of plate 3 was added to a 96-well filter plate. Well A1 of the 96-well plate was applied to B1 of 384-well plate, which was filled with 15  $\mu$ L of Q Hyper D

20  $\mu$ m, lot 7195 and 50 mM Tris pH 7m 500 mM NaCl.

All of each well of plate 4 was added to a 96-well filter plate. Well A1 of the 96-well plate was applied to B2 of 384-well plate, which was filled with 15  $\mu$ L of Q Hyper D 20  $\mu$ m, lot 7195 and 50 mM Tris pH 7m 500 mM NaCl.

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#### Washing

Each well was washed three times with 75  $\mu$ L of 500 mM NaCl, 50 mM Tris pH 8.5. The sample was then spun at 2400 RPM in a Beckman G6 centrifuge for 3 minutes to remove any remaining interstitial liquid.

#### Elution

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Each well was eluted with 30  $\mu$ L of 1M NaCl, 50 mM Tris, pH 8.5 by placing elution buffer in each well, and then placing the first 384-well plate containing the samples on top of a second 384-well plate, wherein each well contained 75  $\mu$ L of a desalting media, GF05 (BioSeptra, Inc.). The desalting plate was preequilibrated with DI water (18 MegaOhm) and was vacuumed and then centrifuged at 2400 RPM for 3 minutes to remove any interstitial water.

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This two plate assembly was then placed on top of a 384-well receiving plate, and the assembly was centrifuged at 2400 RPM for 3 minutes to draw the elution buffer through the Q HyperD media to elute the plasmid, and to then draw the plasmid solution through the GF05 well to desalt it.

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The pUC 19 plasmid was eluted from the plate assembly with an average of 2.82  $\mu$ g per well, with a standard deviation of 0.5 in Row K. Other rows indicated a similar recovery and variability, excluding broken wells previously mentioned. The  $\beta$  gal plasmid was eluted in Row L at 2.08  $\mu$ g with a standard deviation of 0.59  $\mu$ g. These were quantified using a fluorimeter and a dye which has affinity for DNA.

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#### EXAMPLE 2

##### Preparation

RNase (Sigma, St. Louis, Missouri) - In 100 unit increments, increasing amounts of

were added to one 300  $\mu$ L 96-well plate. Water was added to dilute smaller samples to the final volume of 6  $\mu$ L.

The PLASMIDEX plate was prepared with lot 7195, 40  $\mu$ L of 40% slurry, except Row K, which was prepared with 30  $\mu$ L 40% slurry. The plate was washed.

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#### Procedure

About 1 g of cells were resuspended in 200  $\mu$ g/mL RNase resuspension buffer (250 units/mL RNase). 133  $\mu$ L were decanted into each well of two 96-well plates. 6  $\mu$ L of the various concentrations of RNase were added to all the wells of Row D. Two 133  $\mu$ L samples of the resuspended cells were removed for analysis by Qiagen Silica™ or Qiagen IEX™. Standard Qiagen™ lysis of these cells was performed on the side, by adding the appropriate amount of Qiagen™ resuspension buffer to each well and following established protocol. Two samples of 133  $\mu$ L resuspended cells were decanted into a third 96-well culture plate, to be used as a filter standard. Two further samples were removed for standard IPA precipitation for side by side comparison with the filtered only samples.

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The three 96-well plates were lysed according to standard protocols, starting lysis in five minute delays.

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All but one row of each of the two full plates was precipitated with 1.32 M KAc according to standard protocol. Row C of plate 1 and Row C of plate 2, were then precipitated with 1.32 M KAc, 300 mM NaCl. For the two samples in the third 96-well plate, only standard lysis was performed.

The samples from filter plate 1 were drawn through to the 384-well PLASMIDEX plate. The samples from plate 2 were similarly drawn through. Sample A1 of plate 1 goes to A1 and sample A1 of plate 2 goes to A2 for ease of analysis. Row C thus became Row E, Row D became Row G, Row F became Row K, *etc.* as shown in Table 1 below.



Table 1

96-WELL PLATE MAP	384-WELL PLATE MAP		
A	A	standard lysis	standard wash
B	C	standard lysis	3 x 75 $\mu$ L wash
C	E	KAc, 300 mM NaCl	
D	G	RNase series	
E	I	standard lysis	standard wash
F	K	standard lysis	30 $\mu$ L slurry, 12 $\mu$ L bed
G	M	standard lysis	Triton X wash
H	O	standard lysis	4 x 75 $\mu$ L wash

The two samples from the third 96-well culture plate were added to a filter plate and filtered directly into a 96-well collection plate. Standard IPA precipitation was performed to provide a "Filtration Standard." DyNAQuant (Pharmacia BioTech, Uppsala, Sweden) and Gel electrophoresis was used for analysis.

Row M was washed twice with 75  $\mu$ L of 1% Triton X, 500 mM NaCl, 50 mM Tris pH 8.5. The PLASMIDEX was washed twice with 75  $\mu$ L, care was taken to ensure that all empty wells (*i.e.*, Rows B, D, *etc.*) were also washed, for later analysis of well to well contamination. Vacuum was then applied and the PLASMIDEX again washed with 75  $\mu$ L wash. Vacuum was repeated. Washes were collected in a 96-well plate. The wells in row C were then washed with a third 75  $\mu$ L wash. The wells in row O were washed with a third and fourth 75  $\mu$ L wash. Standard IPA precipitation was performed on four of the standard washes and then analyzed with DyNAQuant (Pharmacia BioTech, Uppsala, Sweden).

30  $\mu$ L of elution buffer was added to the wells and neighboring wells having no plasmid in them. The material in the PLASMIDEX plate was then spun through to a GF05 desalting plate. Side by side comparisons of the variables were conducted by gel electrophoresis, DyNAQuant (Pharmacia BioTech, Uppsala, Sweden) and UV (260/280).

In accordance with the present invention, various kits for culturing and purifying a desired product or biological molecule of interest can be assembled. Such kits contain different multiwell plates loaded with various chromatographic media, and any required reagents, such as, for example, a resuspension buffer, a lysis buffer, a potassium acetate ("KAc") precipitation buffer, a washing buffer (e.g., 0.5 M NaCl, 50 mM Tris), a desalting  
5 buffer and/or an elution buffer.

The embodiments of the present invention described above are intended to be merely exemplary and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures and  
10 embodiments described herein. All such equivalents are considered to be within the scope of the present invention and are covered by the following claims.

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### Claims

What is claimed is:

1. A device for purification of biological molecules, the device comprising:  
a first housing having an inlet in an upper surface and an outlet in a lower surface,  
and wherein a porous material is disposed over said outlet;  
a volume of an adsorptive media packed in the first housing, over said porous material and wherein a packed volume of the adsorptive media is 30  $\mu\text{L}$  or less;  
a second housing having an inlet in an upper surface and an outlet in a lower surface, and wherein a porous material is disposed over said outlet; and  
a volume of a second chromatographic media packed in the second housing, over said porous material, and wherein a packed volume of the second media is 100  $\mu\text{L}$  or less;  
wherein the outlet of said first housing is aligned with the inlet of said second housing such that a material exiting the outlet of said first housing passes into the inlet of said second housing.
2. The device of claim 1, wherein the adsorptive media is chosen from an ion exchange media, affinity media, normal phase media, reversed-phase media, metal chelating media, and hydroxyapatite media.
3. The device of claim 1 wherein the second chromatographic media is chosen from a size exclusion media, a silica binding media, a hydrophobic-interaction media, and a reversed-phase media.
4. The device of claim 1 wherein the adsorptive media is an ion exchange media and the second chromatographic media is a desalting media.
5. The device of claim 1, wherein the packed volume of the adsorptive media is less than 15  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 75  $\mu\text{L}$ .

6. The device of claim 3, wherein the packed volume of the adsorptive media is less than 10  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 30  $\mu\text{L}$ .
7. The device of claim 1 wherein the first and the second housings are corresponding wells in separate multiwell plates.
8. The device of claim 1 further comprising a sealing means for forming a seal between said first housing and said second housing.
9. The device of claim 8 wherein the sealing means is one of a gasket disposed on a bottom surface of said first housing or an O-ring disposed on the upper surface of said second housing.
10. A method for purification of biological molecules, the method comprising the steps of:
  - preequilibrating a device of claim 1 with a buffer;
  - loading the preequilibrated device with a biological sample comprised of biological molecules of interest and other impurities, wherein said molecules of interest are adsorbed onto the adsorptive media;
  - washing the loaded device with one or more wash buffers;
  - eluting the molecules of interest from said adsorptive media with a volume of an elution buffer; and
  - collecting the resulting elutant, wherein said elutant comprises the molecules of interest.
11. The method of claim 10 further characterized in that the biological molecules are nucleic acids.
12. The method of claim 10 wherein the volume of elution buffer is less than or equal to one-third the volume of the second chromatographic media.

13. The method of claim 10 further characterized in that the adsorptive media is an ion exchange media and the second chromatographic media is a desalting media.

14. The method of claim 10 further characterized in that the packed volume of the adsorptive media is less than 15  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 75  $\mu\text{L}$ .

15. The method of claim 14 further characterized in that the packed volume of the adsorptive media is less than 10  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 30  $\mu\text{L}$ .

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16. The method of claim 15 wherein the volume of the elution buffer is less than or equal to one-third the volume of the second chromatographic media.

17. The method of claim 10 further characterized in that the first and second housings are corresponding wells in separate multiwell plates.

18. The method of claim 10, wherein the step of eluting comprises introducing said elution buffer into the loaded device and centrifuging the device wherein the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and wherein the resulting elutant is drawn through the second chromatographic media.

19. The method of claim 10, wherein the device further comprises a sealing means for forming a seal between said first and second housings and the step of eluting comprises introducing said elution buffer into the loaded device and applying a pressure differential between an upper surface of the adsorption media and a lower surface of the second media, wherein the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and wherein the resulting elutant is drawn through the second chromatographic media.

20. The method of claim 19 wherein the sealing means is one of a gasket disposed on a bottom surface of the first housing or an O-ring disposed on the upper surface of the second housing.

21. The method of claim 10 wherein the device further comprises a sealing means for forming a seal between said first and second housings and the step of eluting comprises introducing said elution buffer into the loaded device and applying a vacuum at a lower surface of the second media, wherein the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and wherein the resulting elutant is drawn through the second chromatographic media.

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22. The method of claim 21 wherein the sealing means is one of a gasket disposed on a bottom surface of the first housing or an O-ring disposed on the upper surface of the second housing.

23. The method of claim 10 wherein the device further comprises a sealing means for forming a seal between said first and second housings and the step of eluting comprises introducing said elution buffer into the loaded device and applying a first voltage to an upper surface of the adsorption media and a second voltage to a lower surface of the second media, wherein an electro-osmotic flow occurs such that the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and the resulting elutant is drawn through the second chromatographic media.

24. The method of claim 23 wherein the sealing means is one of a gasket disposed on a bottom surface of the first housing or an O-ring disposed on the upper surface of the second housing.

25. A device for purification of biological molecules, the device comprising:  
a housing having an inlet in an upper surface and an outlet in a lower surface, and a porous material disposed over said outlet; and  
a volume of chromatographic media packed in said housing, said chromatographic media comprising:  
a packed volume of 30  $\mu\text{L}$  or less of an adsorptive media and  
a packed volume of 100  $\mu\text{L}$  or less of a second chromatographic media.
26. The device of claim 25, wherein the adsorptive media is chosen from an ion exchange media, affinity media, normal phase media, reversed-phase media, metal chelating media, and hydroxyapatite media.
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27. The device of claim 25 wherein the second chromatographic media is chosen from a size exclusion media, a silica binding media, a hydrophobic-interaction media, and a reversed-phase media.
28. The device of claim 25 wherein the adsorptive media is an ion exchange media and the second chromatographic media is a desalting media.
29. The device of claim 25 wherein the packed volume of the adsorptive media is less than 15  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 75  $\mu\text{L}$ .
30. The device of claim 29 wherein the packed volume of the adsorptive media is less than 10  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 30  $\mu\text{L}$ .
31. The device of claim 25 wherein the housing is a well of a multiwell plate.
32. A method for purification of biological molecules, the method comprising the steps of:  
preequilibrating a device of claim 25 with a buffer;  
loading the preequilibrated device with a biological sample comprised of biological

molecules of interest and other impurities, wherein said molecules of interest are adsorbed onto the adsorptive media;

washing the loaded device with one or more wash buffers;

eluting the molecules of interest from said adsorptive media with a volume of an elution buffer; and

collecting the resulting elutant, wherein said elutant comprises the molecules of interest .

33. The method of claim 32 further characterized in that the biological molecules are nucleic acids.

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34. The method of claim 32 further characterized in that the volume of elution buffer is less than or equal to one-third the volume of the second chromatographic media.

35. The method of claim 32 further characterized in that the adsorptive media is an ion exchange media and the second chromatographic media is a desalting media.

36. The method of claim 32 further characterized in that the packed volume of the adsorptive media is less than 15  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 75  $\mu\text{L}$ .

37. The method of claim 36 further characterized in that the packed volume of the adsorptive media is less than 10  $\mu\text{L}$  and the packed volume of the second chromatographic media is less than 30  $\mu\text{L}$ .

38. The method of claim 37 wherein the volume of the elution buffer is less than or equal to one-third the volume of the chromatographic media.

39. The method of claim 32, further characterized in that the housing is a well of a multiwell plate.

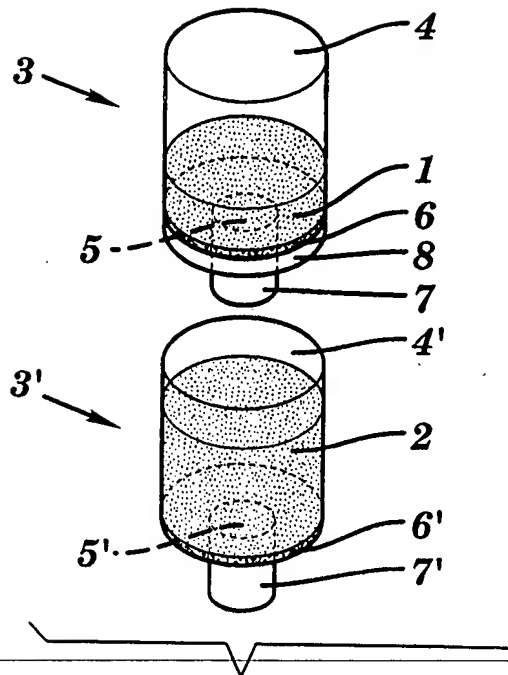
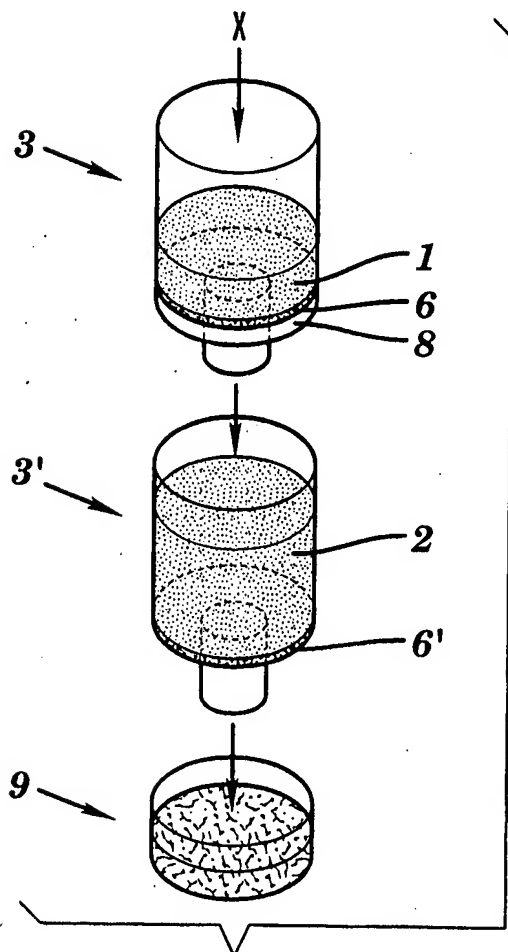


40. The method of claim 32, wherein the step of eluting comprises introducing said elution buffer into the loaded device and centrifuging the device wherein the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and wherein the resulting elutant is drawn through the second chromatographic media.

41. The method of claim 32 wherein the step of eluting comprises introducing said elution buffer into the loaded device and applying a pressure differential between an upper surface of the adsorption media and a lower surface of the second chromatographic media, wherein the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and wherein the resulting elutant is drawn through the second chromatographic media.

42. The method of claim 32 wherein the step of eluting comprises introducing said elution buffer into the loaded device and applying a vacuum at a lower surface of the second chromatographic media, wherein the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and wherein the resulting elutant is drawn through the second chromatographic media.

43. The method of claim 32 wherein the step of eluting comprises introducing said elution buffer into the loaded device and applying a first voltage to an upper surface of the adsorption media and a second voltage to a lower surface of the second chromatographic media, wherein an electro-osmotic flow occurs such that the elution buffer is drawn through the adsorptive media, eluting said molecules of interest and the resulting elutant is drawn through the second chromatographic media.

**FIG. 1****FIG. 2**

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/23997

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 B01D15/08 B01L3/00 C12M1/16 G01N30/60 G01N30/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 B01D B01L C12M G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 598 933 A (LESSARD DENIS ET AL) 4 February 1997	1-9, 25-31
A	see column 9, line 7-65; figures 1,19	10-24, 31-43
X	DE 42 08 732 A (ABION OHG) 30 September 1993 see column 2, line 27-37; figure 1A see column 3, line 5-28	25-31
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

23 February 1999

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PCT/US 98/23997

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